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## Hot topic: Innovative lactation-stage-dependent prediction of methane emissions from milk mid-infrared spectra

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### ABSTRACT

The main goal of this study was to develop, apply, and validate a new method to predict an indicator for CH<sub>4</sub> eructed by dairy cows using milk mid-infrared (MIR) spectra. A novel feature of this model was the consideration of lactation stage to reflect changes in the metabolic status of the cow. A total of 446 daily CH<sub>4</sub> measurements were obtained using the SF<sub>6</sub> method on 142 Jersey, Holstein, and Holstein-Jersey cows. The corresponding milk samples were collected during these CH<sub>4</sub> measurements and were analyzed using MIR spectroscopy. A first derivative was applied to the milk MIR spectra. To validate the novel calibration equation incorporating days in milk (DIM), 2 calibration processes were developed: the first was based only on CH<sub>4</sub> measurements and milk MIR spectra (independent of lactation stage; ILS); the second included milk MIR spectra and DIM information (dependent on lactation stage; DLS) by using linear and quadratic modified Legendre polynomials. The coefficients of determination of ILS and DLS equations were 0.77 and 0.75, respectively, with standard error of calibration of 63 g/d of CH<sub>4</sub> for both calibration equations. These equations were applied to 1,674,763 milk MIR spectra from Holstein cows in the first 3 parities and between 5 and 365 DIM. The average CH<sub>4</sub> indicators were 428, 444, and 448 g/d by ILS and 444, 467, and 471 g/d by DLS for cows in first, second, and third lactation, respectively. Behavior of the DLS indicator throughout the lactations was in agreement with the literature with values increasing between 0 and 100 DIM and decreasing thereafter. Conversely, the ILS indicator of CH<sub>4</sub> emission decreased at

the beginning of the lactation and increased until the end of the lactation, which differs from the literature. Therefore, the DLS indicator seems to better reflect biological processes that drive CH<sub>4</sub> emissions than the ILS indicator. The ILS and DLS equations were applied to an independent data set, which included 59 respiration chamber measurements of CH<sub>4</sub> obtained from animals of a different breed across a different production system. Results indicated that the DLS equation was much more robust than the ILS equation allowing development of indicators of CH<sub>4</sub> emissions by dairy cows. Integration of DIM information into the prediction equation was found to be a good strategy to obtain biologically meaningful CH<sub>4</sub> values from lactating cows by accounting for biological changes that occur throughout the lactation.

**Key words:** dairy cattle, mid-infrared, methane, lactation stage, milk

### Hot Topic

Feed costs are the largest expense in dairy production and limit the profitability of dairy farms (Vallimont et al., 2011). Methane emissions, besides contributing to the carbon footprint of dairy products, represent a loss of use of energy intake (Johnson and Johnson, 1995). Therefore, interest is growing in mitigating CH<sub>4</sub> emissions, thereby reducing environmental footprint but also potentially improving feed efficiency and reducing related feed costs. Most of the CH<sub>4</sub> emitted by cattle is eructed. However, the measurement of eructed CH<sub>4</sub> emissions is difficult and expensive. To mitigate these emissions, it is important to have a cheap, fast, and robust method to measure the CH<sub>4</sub> emissions of individual cows on a large scale. Traits that can be easily recorded and that are correlated with CH<sub>4</sub> emissions are therefore of interest. De novo synthesis of milk FA in the mammary gland is dependent on the products of

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ruminal fermentation (Chilliard et al., 2000), as is the production of eructed  $\text{CH}_4$  (Chilliard et al., 2009; Dijkstra et al., 2011). Hence, changes in the FA profile of milk fat may be relevant. Because mid-infrared (MIR) spectrometry is known to be useful in predicting milk FA contents (Soyeurt et al., 2011), Dehareng et al. (2012) developed prediction equations to obtain individual  $\text{CH}_4$  emissions of dairy cows directly from milk MIR spectra. However, results obtained using those equations did not fit with what was expected from the literature, showing the lowest emissions in early lactation and an increase thereafter. A hypothesis could be that milk FA profile, and therefore the relationship between  $\text{CH}_4$  and MIR spectra, is strongly influenced by the evolution of body-tissue mobilization during lactation, leading to suboptimal predictions. Consequently, MIR prediction equations that incorporate stage of lactation may better take into account the metabolic changes that occur throughout lactation. The objective of this study was to develop, apply, and test on MIR data, a novel and readily adoptable method that includes lactation stage as a proxy for metabolic status in the  $\text{CH}_4$  prediction equation based on milk MIR spectra. The ability of the novel method to predict a  $\text{CH}_4$  indicator was tested on a completely unrelated external data set.

### Development of Mid-Infrared-Based $\text{CH}_4$ Indicators

**Methane Reference Measurements.** Measurements of  $\text{CH}_4$  emissions from dairy cows were obtained from the Teagasc Animal and Grassland Research and Innovation Center (Moorepark, Co. Cork, Ireland), the Walloon Agricultural Research Centre (Gembloux, Belgium), and from one commercial herd in Belgium. A total of 446 individual daily  $\text{CH}_4$  measurements were recorded: 270 Irish records from 117 individual Holstein, Jersey, and Holstein-Jersey crossbred cows, including 49 first-, 30 second-, and 38 third- or later-parity animals; and 176 Walloon records from 25 Holstein cows including 10 first-, 6 second-, and 9 third- or later-parity animals. Measurements were conducted at

different lactation stages (Table 1). This data set of  $\text{CH}_4$  values was characterized by a minimum of 180 g/d, a maximum of 942 g/d, a mean of 416 g/d, and a standard deviation of 128 g/d. To achieve the largest possible variability required for robust calibration equations, cows were fed with very different diets: grass or high silage diets (maize vs. grass silages), with or without linseed supplementation, and synchronized or not in terms of fermentable energy and nitrogen supplies in the rumen. Daily  $\text{CH}_4$  emissions of individual cows were determined using the sulfur hexafluoride ( $\text{SF}_6$ ) tracer gas technique with a gas collection period of 24 h. Details about the methodology used to collect the breath samples of emitted gases and to measure the  $\text{SF}_6$  and the  $\text{CH}_4$  content were described by Dehareng et al. (2012). By using  $\text{CH}_4$  emission data collected from different countries, environments, and feeding strategies, an increase in the variability of  $\text{CH}_4$  emission was expected, ensuring improved robustness of the equation developed to predict  $\text{CH}_4$  emissions.

**Spectral Reference Data.** On each day of the  $\text{CH}_4$  measurements, a 40-mL aliquot of milk was collected from each cow at both the morning and evening milking. Sodium azide (0.32 g/L) was added to each sample before storage at 4°C and MIR analysis was subsequently undertaken using a MilkoScan FT6000 spectrometer (Foss, Hillerød, Denmark). Two spectrometers were used for this study, one located at the Teagasc Animal and Grassland Research and Innovation Center (Co. Cork, Ireland) and one at the milk laboratory Comité du Lait (Battice, Belgium). Two spectra were available per day and per  $\text{CH}_4$  measurement. To obtain 1 spectrum per  $\text{CH}_4$  record, the 2 spectra were averaged proportionally to the milk yield at each milking.

**Development of  $\text{CH}_4$  Indicator Equations.** To determine the effect of inclusion of DIM information as a predictor variable, 2 prediction equations were developed using independent calibration processes;  $\text{CH}_4$  was the dependent variable for both equations. The first prediction equation was independent of lactation stage (ILS) and included milk MIR spectra as the sole pre-

**Table 1.** Distribution of the number of records in the calibration data set, by parity and stage of lactation of cows, and country from where records were obtained, used to develop methane emissions indicators

Classes of DIM	Parity					
	First		Second		Third or greater	
	Ireland	Belgium	Ireland	Belgium	Ireland	Belgium
1 to 50 DIM	1	7	1	0	4	0
51 to 100 DIM	19	8	21	16	31	0
101 to 150 DIM	31	13	19	10	27	15
151 to 200 DIM	46	19	18	6	12	3
>200 DIM	19	33	13	6	8	40

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